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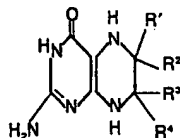
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Mauerkircherstrasse 45, D-8000 München 80 (DE)(54) **Pharmaceutically active pteridine derivatives.**

(57) The use of compounds of the formula (I):



R³ and R⁴ can all be hydrogen, in the treatment of neurological disorder is disclosed. Pharmaceutical compositions containing compounds of the formula (I), novel compounds and processes for preparing the novel compounds are also disclosed.

or pharmaceutically acceptable salts or bio-precursors thereof, wherein

R¹ is hydrogen, C₁₋₄ alkyl, C₂₋₄ alkenyl, a group -BX(R⁵)_n, or -BZBX(R⁵) wherein Z and X (when n = 1) are the same or different and each is -O, -S(O)q or -NR⁶, wherein q is 0, 1 or 2 and R⁶ is hydrogen or C₁₋₄ alkyl or, when n = 0, X is halogen;

R² is hydrogen, C₁₋₁₂ aralkyl or C₁₋₁₀ alkyl;

B is C₁₋₆ alkanyl;

R³ is hydrogen, C₁₋₄ alkyl, C₂₋₄ alkenyl, or a group BOR⁷

wherein R⁷ is hydrogen or C₁₋₆ alkyl;

or R¹ and R² together with the carbon atoms in the pteridine ring structure to which they are attached form a C₅₋₇ cycloalkyl ring;

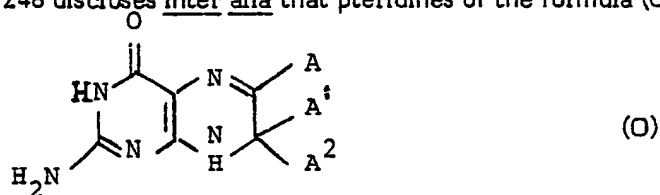
provided that at least one of R¹, R², R³, and R⁴ is hydrogen and that one of R¹, R², and R³, R⁴ represents gem di-substitution unless R¹ represents CH₂OR⁷ in which case R²,

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CHEMICAL COMPOUNDS

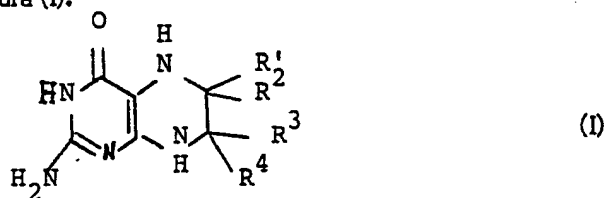
The present invention relates to a series of pteridines known as pterins which are analogs of tetrahydrobiopterin, to pharmaceutical formulations containing them, to processes for their preparation and to the use thereof in human medicine. More specifically the invention relates to certain biopterin analogs and their use in the treatment of Parkinsonism (and other diseases caused by a deficiency of catecholamines in the brain and the peripheral nervous system) and the tetrahydrobiopterin-deficient phenylketonurias.

UK Patent No 1405 246 discloses inter alia that pteridines of the formula (O):



wherein A is a C_{1-4} alkyl group optionally substituted by a hydroxy group or a halogen atom; and A^1 and A^2 are the same or different and each is a C_{1-4} alkyl group. These compounds are inhibitors of the enzyme hydroxymethyldihydropteridine pyrophosphokinase and thereby inhibit the growth of microorganisms and, in addition, potentiate the anti-microbial activity of a competitor of p-aminobenzoic acid and/or an inhibitor of dihydrofolate reductase. No CNS activity of these compounds is disclosed. It has now been found that the corresponding tetrahydro pteridines are useful in the treatment of neurological disorders.

Accordingly, the present invention provides the first medical use of pterin compounds of the formula (I):



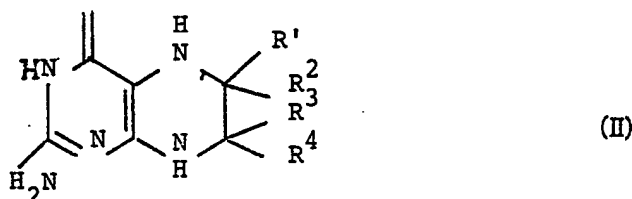
or a pharmaceutically acceptable salt or a bio-precursor thereof;
wherein R^1 is hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, a group $-BX(R^5)_n$, or $-BZ BX-(R^5)_n$ wherein Z and X (when $n=1$) are the same or different and each is $-O-$, $-S(O)_q$ or $-NR^6$ -wherein q is 0, 1 or 2 and R^6 is hydrogen or C_{1-4} alkyl or when $n=0$, X is halogen; R^5 is hydrogen, C_{1-12} aralkyl or C_{1-10} alkyl; B is C_{1-5} alkanyl;



R^2 is hydrogen C_{1-4} alkyl, C_{2-4} alkenyl or a group BOR^7 wherein B is as hereinbefore defined and R^7 is hydrogen or C_{1-6} alkyl; or R^1 and R^2 together with the carbon atom in the pteridine ring structure to which they are attached, form a C_{3-7} spirocycloalkyl ring;
 R^3 and R^4 are hydrogen or methyl;
 or R^2 and R^3 together with the carbon atoms in the pteridine ring structure to which they are attached form a C_{5-7} cycloalkyl ring;
 provided that at least one of R^1 , R^2 , R^3 and R^4 is hydrogen and that one of R^1 , R^2 and R^3 , R^4 represents gem di-substitution unless R^1 represents $-CH_2OR^7$ wherein R^7 is as hereinbefore defined in which case R^2 , R^3 and R^4 can all be hydrogen.

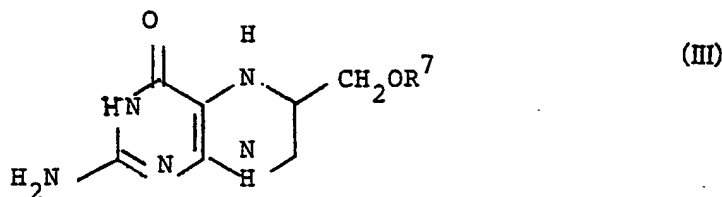
When used herein, and where appropriate, the terms alkyl, alkenyl and alkanyl cover straight, branched and cyclic chains.

A preferred group of compounds of the formula (I) is that of the formula (II):



or a pharmaceutically acceptable salt or bioprecursor thereof; wherein R^1 to R^4 are as hereinbefore defined and at least one of R^1 , R^2 and R^3 , R^4 represents gem di-substitution.

A further preferred group of compounds of the formula (I) is that of the formula (III):



or a pharmaceutically acceptable salt or bioprecursor thereof;
wherein R^7 is as hereinbefore defined.

In a further aspect, the present invention provides novel pterin compounds of the formula (I) or pharmaceutically acceptable salts thereof, that is to say the compounds of the formula (I) as hereinbefore defined with the additional proviso that when both R^3 and R^4 are methyl neither R^1 nor R^2 are methyl.

A preferred group of novel compounds of the formula (I) is that of the formula (II) or a pharmaceutically acceptable salt thereof; wherein formula (II) is as hereinbefore defined with the additional proviso that when R^3 and R^4 are methyl neither R^1 nor R^2 are methyl.

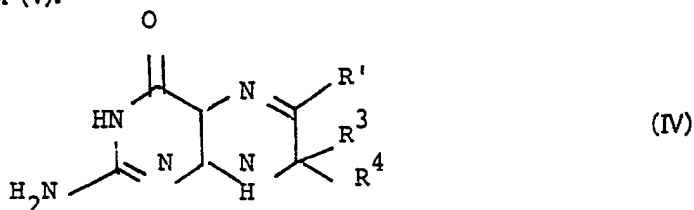
A further preferred group of novel compounds of the formula (I) is bioprecursors of compounds of the formula (III) as hereinbefore defined.

Suitably R^1 is hydrogen, C_{1-4} alkyl or a group $-BX(R^5)_n$ as hereinbefore defined. Suitably B is $-\text{CH}_2-$, X is oxygen and $(R^5)_n$ represents hydrogen or a C_{1-10} alkyl group, preferably hydrogen a C_{1-6} alkyl group. Most suitably R^1 is hydrogen, methyl, ethyl or a group $-\text{CH}_2\text{O}(R^5)_n$ wherein R^5 represents hydrogen or a C_{1-6} alkyl group, such as a methyl or butyl group. Preferably R^1 is a group $-\text{CH}_2\text{O}(R^5)_n$ wherein R^5 is hydrogen or a C_{1-6} alkyl group.

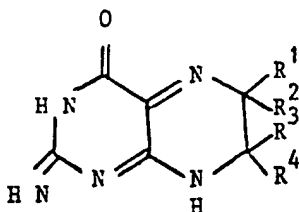
Suitably, R^2 is a hydrogen atom or a C_{1-6} alkyl group. Preferably R^2 is a hydrogen atom.

Suitably, at least one of R^3 and R^4 is methyl.

The compounds of the formula (I) may be formed enzymatically from compounds of the formula (IV) or (V):



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(V)

wherein R¹ to R⁴ are as hereinbefore defined, by the action of dihydrofolate reductase and dihydropteridine reductase respectively. As these enzymes are present in mammals, the compounds of the formulae (IV) and (V) are bioprecursors of the compounds of the formula (I) and the term bioprecursor when used herein relates to these compounds.

Preferred compounds of the formula (I) include (+)-2-amino-5,6,7,8-tetrahydro-6-methoxymethyl-4(3H)-pteridinone and (+)-2-amino-5,6,7,8-tetrahydro-6-n-butoxymethyl-4(3H)-pteridinone or pharmaceutically acceptable salts thereof.

The compounds of the formula (I) contain assymetric carbon atoms and the compounds may therefore be obtained in different isomeric forms and as mixtures of isomeric forms. The present invention includes within its scope the different isomeric forms and mixtures of the forms.

It is generally accepted that certain substances known as neurotransmitters are required at the microscopic regions, known as synapses, between nerve cells (neurons) to transmit the nerve impulses throughout the body.

Over thirty substances are known or suspected to be neurotransmitters and each has a characteristic excitatory or inhibitory effect on neurons. Excesses or deficiencies of these transmitters can be manifested as moderate to severe neurological or mental disorders. Whereas neurons are present throughout the body, imbalances of neurotransmitters at synapses of the neurons in the brain are by far the most critical and produce the most pronounced effects.

Of the numerous neurotransmitters known or thought to be operating at synapses, a smaller group collectively known as biogenic amines have received the most study. Particularly important members of this group are the catecholamines such as dopamine and norepinephrine (noradrenaline) and the indole amine, serotonin.

Tetrahydrobiopterin (BH₄) is an essential cofactor for the rate limiting enzymes of biogenic amine synthesis, tyrosine and tryptophan hydroxylases, and for the liver enzyme which converts phenylalanine to tyrosine (Kaufman, S. and Fisher, D. B., Molecular Mechanisms of Oxygen Activation, Hayaishi, O. Ed., Acad. Press, N.Y.

(1974)). Knowledge of the chemical pathology of neurological disorders has expanded tremendously during the last two decades. For example, neurological disorders have been described whose symptoms can be associated with decreases in the number of catecholamine molecules released at certain synaptic sites. As a category, they may be thought of as 'catecholamine-deficiency disorders'. One example is Parkinson's disease (also known as Parkinsonism), where a deficiency in brain dopamine has been linked with the symptoms of rigidity, tremor and akinesia. Another example is chronic preganglionic autonomic insufficiency known as the Shy-Drager syndrome which is associated with both peripheral sympathetic dysfunction and a degeneration of brain neurons in the basal ganglia. The peripheral sympathetic dysfunction most likely reflects a loss of formation and release of the pressor catecholamine norepinephrine, while the rigidity and akinesia most likely reflect a loss of the capacity to form dopamine in certain brain regions.

In all of these cases the catecholamines, whose levels are diminished, are formed through the action of tyrosine hydroxylase which is rate-limiting for their formation. This enzyme requires tyrosine, oxygen and a reduced pterin cofactor, tetrahydrobiopterin (BH_4), for activity. While oxygen and tyrosine are not normally limiting for tyrosine hydroxylase, the levels of BH_4 may normally be well below the levels required to saturate this enzyme. In fact, there are reports in the scientific literature indicating the levels of this cofactor are severely diminished in Parkinson's disease and in the Shy-Drager syndrome (Nagatsu, T., *Neurochem. Intern.*, 5, 27 (1983)). Logically, the rate of dopamine biosynthesis would be increased by reversing this deficit. Administration of BH_4 has been shown to nearly double striatal dopamine synthesis, as well as noradrenaline synthesis in peripheral nerves (Nagatsu, vide supra also Cloutier, G and Weiner, N., *J. Pharm. Exp. Ther.*, 186, 75 (1973)). In fact, BH_4 administration has been reported to reduce the symptoms of Parkinson's disease (Narabayashi, H., Kond, T., Nagatsu, T., Sugimoto, T. and Matsuura, S., *Proc. Japan Acad.*, 58, Ser. B, 283 (1982)). A group of psychiatric disorders known as endogenous depression may also involve reduced neuronal formation of catecholamines and serotonin. In fact, BH_4 has recently been shown to reduce the symptoms of depression in several patients (Curtius, H., Muldner, H. and Niederwieser, A., *J. Neurol. Transmission*, 55, 301 (1982)). This natural cofactor is, however, expensive and unstable, and it penetrates brain poorly. Previously known synthetic cofactor replacements for BH_4 all cause tyrosine hydroxylase (TH) to bind its substrate tyrosine more weakly. Thus, the administration of these previously known synthetic cofactors for the acceleration of dopamine biosynthesis in the treatment of catecholamine deficiency causes TH to become unsaturated with tyrosine.

Compounds of formula (I), quite unexpectedly, have been found not to decrease the binding of tyrosine to tyrosine hydroxylase and to promote dopamine formation by this enzyme at rates equal to that observed with the natural cofactor, BH_4 . Further, certain compounds of formula I enter the brain more easily than BH_4 and accelerate dopamine biosynthesis better than the natural cofactor when administered peripherally (orally). Thus it is believed that the administration of compounds of formula (I) for catecholamine deficiency will accelerate dopamine biosynthesis without co-administration of exogenous tyrosine.

Endogenous (psychotic) depression is thought to involve decreased levels of serotonin and noradrenaline at brain synapses. Certain compounds of the formula (I) (those of the formula (II)), function as cofactors for tyrosine hydroxylase but not tryptophan hydroxylase. These compounds have a selective effect therefore when compared with BH_4 .

Certain other compounds of the formula (I), the compounds of the formula (III) function as cofactors for tyrosine and tryptophan hydroxylases. Thus, these compounds will reduce the symptoms of endogenous depression by promoting the synthesis of both catecholamines and serotonin in the brain. In addition to actions on the central nervous system, the compounds of formula (III) can replace BH_4 in the liver and promote the hydroxylation of phenylalanine.

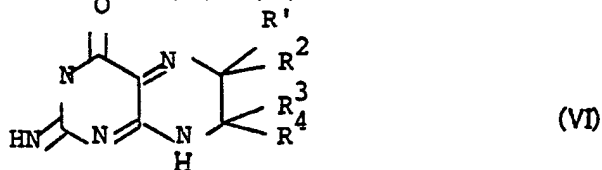
Indeed, a small percentage of all patients with phenylketonuria suffer from a tetrahydrobiopterin-deficient form of phenylketonuria. This "atypical PKU" has been successfully treated with large quantities of BH_4 (Nagatsu, vide supra). Since these compounds of formula (III) are more lipophilic and have better bioavailability than BH_4 , considerably smaller doses could be used to treat this atypical PKU. Thus, it is believed that the compounds of formula (III) have at least 2 benefits. First, they may act as cofactors for hepatic phenylalanine hydroxylase to reduce plasma phenylalanine levels. Second, they may act within the brain as cofactors for tyrosine and tryptophan hydroxylase, to correct the BH_4 deficient reductions in cerebral catecholamine and serotonin levels which are also seen in atypical PKU. Paradoxically, large amounts of phenylalanine inhibit phenylalanine hydroxylase with BH_4 as cofactor. In contrast, compounds of formula (III) have not shown this substrate inhibition and may be expected to remain effective in the presence of large circulating levels of phenylalanine.

Tetrahydropterins, upon reacting with tyrosine, tryptophan or phenylalanine hydroxylases, give rise to an unstable quinoid intermediate which is reduced back to the tetrahydro form by dihydropteridine reductase (DHPR). Compounds of formula (I) have been found to be recycled by DHPR at rates three times faster than that of the natural cofactor. This feature would extend the useful biological life of these compounds, resulting in a favourable duration of action.

The compounds of formula (I) and their salts may be synthesized by methods known in the art for synthesis of compounds having analogous structures.

The compounds of the formula (I) may be prepared by the reduction of the

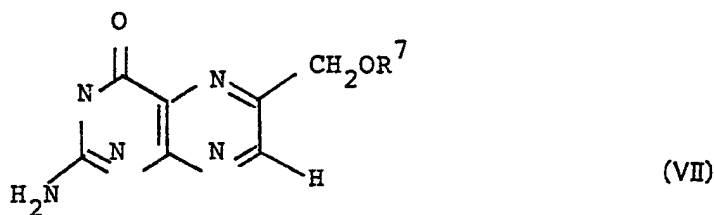
corresponding compound of the formula (IV) or (VI):



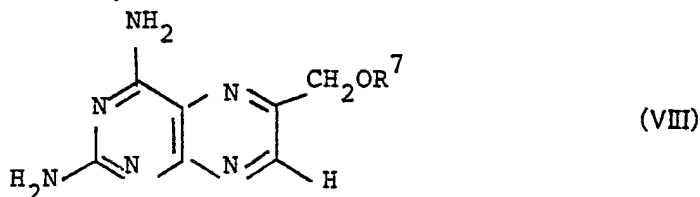
This reduction may be carried out enzymatically (particularly in the case of a compound of the formula (IV)) by use of an appropriate enzyme, such as dihydrofolate reductase, (or dihydropteridine reductase in the case of a compound of the formula (VI)),

or by use of an appropriate reducing agent capable of donating hydrogen, such as hydrogen in the presence of a catalyst, particularly a transition metal catalyst such as platinum oxide or palladium, or a borohydride such as sodium borohydride or sodium cyanoborohydride in an appropriate solvent. Suitable solvents include dilute aqueous acids, such as hydrochloric and trifluoroacetic acids. The reaction is suitably carried out at a non-extreme temperature, i.e. between 0° and 100°C and conveniently at room temperature.

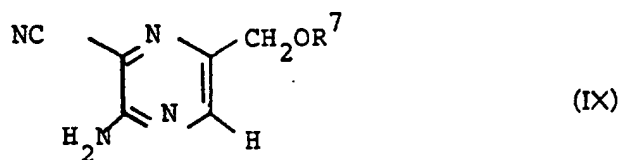
The compounds of the formula (IV) may be prepared by methods well known to those skilled in the art (see for example UK Patents 1303171, 1363064 and 1454165 and Aust. J. Chem., 34, 1921-33, scheme 1 illustrates various methods by which compounds of the formula (IV) may be prepared). The compounds of the formula (IV) wherein R¹ is a group -CH₂OR⁷ as hereinbefore defined may be prepared by reduction of the corresponding pteridinone of the formula (VII) (suitably the pteridinone is reduced in situ directly to the compound of the formula (I) via the compound of the formula (IV)). The pteridinone (VII):



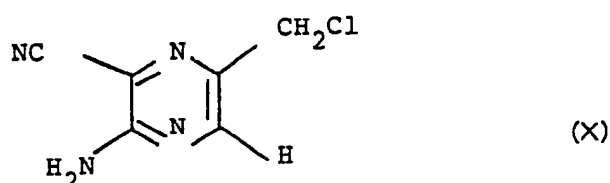
may be prepared by hydrolysis of a compound of formula (VIII) with a suitable agent such as an aqueous sodium hydroxide solution.



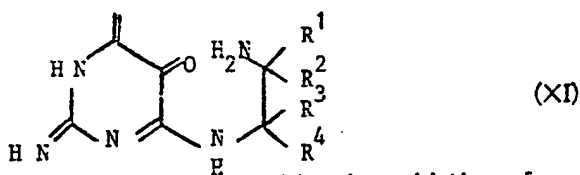
In turn, compounds of formula (VIII) may be prepared by condensation of the compound of formula (IX) with guanidine in the presence of a base, for example sodium alkoxide in an alcohol. A specific example is sodium methoxide in methanol.



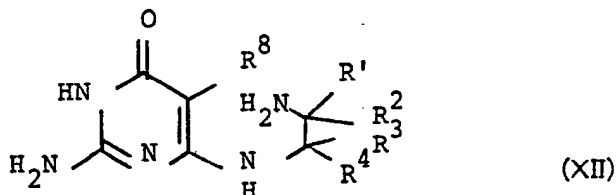
Compounds of formula (IX) may be prepared by refluxing a compound of formula (X) in an alcohol R^7 OH.



Compounds of the formula (VI) may be prepared by cyclisation of compounds of the formula (XI).



The compounds of the formula (XI) may be formed by the oxidation of compounds of the formula (XII):



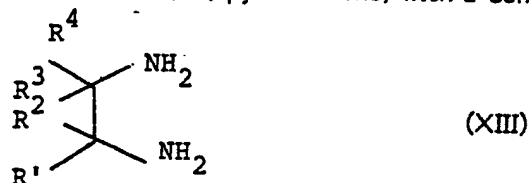
wherein R^1 to R^4 are as hereinbefore defined and R^8 is an amino group

The compounds of the formula (VI) are conveniently formed from the compounds of the formula (XII) without the isolation of the compounds of the formula (XI)

The oxidation of the compounds of the formula (XII) wherein R^8 is an amino group suitably takes place by the addition of bromine to a solution of a compound of the formula (XII) in methanolic trifluoroacetic acid. Neutralisation, after the reaction is complete, leads to the cyclisation of the compounds of the formula (XI) to give the compounds of the formula (VI). When R^1 and R^2 are hydrogen, the compounds of the formula (VI) may rearrange to the corresponding compounds of the formula (IV).

The compounds of the formula (XII) wherein R^8 is an amino group are formed by reduction

of the corresponding compounds of the formula (XII) wherein R^8 is a nitro group. This reduction may be carried out by catalytic hydrogenation or by dithionite. The compounds of the formula (XII) wherein R^8 is a nitro group may be prepared by the condensation of 2-amino-6-chloro-5-nitro-4(3H)-pyrimidinone (formed by the nitration of 2-amino-6-chloro-4(3H) pyrimidinone) with a compound of the formula (XIII):



This condensation reaction is suitably carried out at an elevated temperature in an alkanol, such as ethanol.

The compounds of the formula (I) may also be prepared by the reaction of the formula (IV) with a compound $R^2\text{Li}$. It may be advisable to prevent anion formation in the compound of the formula (XIV) by reaction with a suitable protecting agent, for example a silylating agent, before the reaction with the compound $R^2\text{Li}$. The reaction with $R^2\text{Li}$ may conveniently be carried out in an inert aprotic solvent such as an ether, for example diethyl ether, at between -15° and 50°C , for example at room temperature.

It is believed that compounds of this invention may be used to treat Parkinson's disease, endogenous depression, orthostatic hypotension, muscular dystonia and other disorders which arise from deficiencies of catecholamines and serotonin at the pre-synaptic sites of neuronal junctions. It is also believed that these compounds may be used to treat the BH_4 -deficient phenylketonurias.

The amount of the active compound, i.e. a compound of formula (I), required for use in the above disorders will, of course, vary with the route of administration, the condition being treated, and the person undergoing treatment, but is ultimately at the discretion of the physician. However, a suitable dose for treating these disorders is in the range of from 0.5 to 20 mg per kilogram body weight per day preferably from 1 to 10 mg/kg body weight per day, most preferably from 2 to 7 mg/kg body weight per day, a typical preferred dose in 5 mg/kg body weight per day.

The desired dose is preferably presented as between one and four subdoses administered at appropriate intervals throughout the day. Thus where three subdoses are employed each will lie in the range of from 0.17 to 6.7 mg/kg body weight; a typical dose for a human recipient being 1.7 mg/kg body weight.

If desirable, the catecholamine and serotonin precursors L-tyrosine or L-tryptophan may be administered concurrently with a compound of formula (I) at the rate of 25mg/kg to 1000 mg/kg body weight. These amino acids may be given in the same pharmaceutical formulation, e.g., tablet or capsule, as a compound of formula (I) or in a separate pharmaceutical formulation.

While it is possible for the active compound or compounds to be administered alone as the raw chemicals, it is preferable to present the active compound or compounds as pharmaceutical formulations. Formulations of the present invention comprise a compound of formula (I) together with one or more pharmaceutically acceptable carriers thereof and optionally any other active therapeutic ingredients.

The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The carrier may contain a preservative agent such as ascorbic acid.

The formulations include those suitable for oral, rectal or parenteral (including subcutaneous, intramuscular and intravenous) administration.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a finely divided solid carrier and then, if necessary, shaping the product into the desired formulations or packaging in a suitable container.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active compound(s); as a powder or granules; or a suspension in a non-aqueous liquid such as a syrup, an elixir, and emulsion or a draught. The active compound(s) may also be presented as a bolus or depot formulation.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active compound being in a free-flowing form such as powder

or granule, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, comprising a mixture of the powdered active compound(s) with any suitable carrier.

Formulations for rectal administration may be presented as a suppository with a usual carrier such as cocoa butter.

Formulations suitable for parenteral administration can be made sterile and packed as dry solids in sealed sterile nitrogen-purged containers. At the time of parenteral administration a specified amount of sterile water is added to the drug formulation and the resulting solution is administered to the patient with a minimum of delay since the compounds of formula (I) tend to be unstable in aqueous solution over a period of time.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more accessory ingredient(s) selected from diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like.

Compounds of the formula (I) are particularly useful in increasing the synthesis of tyrosine, dopamine, norepinephrine and serotonin in mammals such as rats and humans. Such effects are produced by administration preferably orally or parenterally.

The following examples are provided by the way of illustration of the present invention and should in no way be construed as a limitation thereof.

Example 1: (+-)-2-Amino-5,6,7,8-tetrahydro-6-methoxymethyl-4(3H)-pteridinone

2-Amino-3-cyano-5-chloromethyl pyrazine (0.95g) was reacted with a large excess of dry methanol, under nitrogen, at reflux, for about 18 hours. The resulting reaction mixture was filtered and the filtrate was taken to dryness by evaporation. A yellow residue was collected and extracted twice with boiling methylene chloride. These extracts were filtered and the solvent evaporated to give 2-amino-3-cyano-5-methoxymethylpyrazine which was then treated with an excess of guanidine in methanol according to the method of Taylor (J. Org. Chem., 2817, 38, (1973)). Most of the product of this reaction precipitated and was collected by

filtration. The filtrate was mixed with silica gel, stripped of solvent by evaporation, and the residue was placed on a silica gel column. This column was eluted with methanol (5%) in ethylacetate to yield a second crop of product which was added to that previously collected. The combined crops were recrystallized from methanol to yield 2,4-diamino-6-methoxymethyl pteridine (mp 248-251⁰).

The diaminopteridine, vide supra, was hydrolyzed in 1N NaOH at 70⁰C for 3 hr. The resulting basic solution was then acidified with acetic acid, and the yellow product was filtered, washed with 95% ethanol and ethyl ether, and dried under vacuum overnight to yield 0.5 g (83%) of 2-amino-6-methoxymethyl-4(3H)pteridinone as a yellow powder: UV (0.1N NaOH) λ_{\max} 255 nm (22300), 277.5 nm sh (6200), 364 nm (7100); NMR (DMSO-d₆) δ 3.36 (s, OMe), 4.53 (s, CH₂O), 7.32 (brs, NH₂), and 8.63 (s, 7-H). Anal. calcd. for C₈H₉N₅O₂ · 7/10H₂O: C, 43.72; H, 4.77; N, 31.86. Found: C, 43.77; H, 4.77; N, 31.63.

The pteridinone (0.5 g, 2.4 x 10⁻³ mol) previously prepared, vide supra, was hydrogenated under 1 atm of H₂ in 30mL of distilled trifluoroacetic acid over 0.02 g of PtO₂ for an hour. The mixture was diluted with 25 mL of 6N HCl, filtered, and evaporated. The crude product, the tetrahydro compound, was recrystallized twice from 6N HCl/acetonitrile to give 2-amino-5,6,7,8-tetrahydro-6-methoxymethyl-4(3H)-pteridinone as a light brown solid (0.6 g, 85%): UV (0.1N HCl) λ_{\max} 215.5 nm (19800), 264 nm (19900); calcd. for C₈H₁₃N₅O₂ HCl · 1/2 H₂O: C, 32.78; H, 5.50; N, 23.89; Cl, 24.19. Found: C, 32.75; H, 5.54; N, 23.80; Cl, 24.21.

Example 2 (+)-2-Amino-5,6,7,8-tetrahydro-6-n-butoxymethyl-4(3H)-pteridinone

This compound was made in a manner similar to that of the 6-methoxymethyl compound described in Example I (vide supra) except for the two following major exceptions: the n-butoxymethylpyrazine was made by heating the chloromethylpyrazine VI in n-butanol instead of methanol, and the guanidine reaction was carried out in heated n-butanol instead of refluxing methanol.

Spectra and microanalytical data of the three intermediates and final product are the following:

2-Amino-3-cyano-5-n-butoxymethylpyrazine. NMR (Me₂SO-d₆) δ 0.89 (t, 3H), 1.1-1.8 (m, 4H), 3.43 (t, 2H), 4.35 (s, 2H), 7.22 (brs, 2H), 8.25 (s, 1H); IR (CHCl₃) 3694,

3526, 3414, 2930, 2866, 2220 (CN), 1723, 1612, 1463, 1377, 1275, 1088 cm^{-1} .

2,4-Diamino-6-n-butoxymethylpteridine. mp 237-238°C dec; NMR ($\text{Me}_2\text{SO}-d_6$) δ 0.87 (t, 3H), 1.0-1.7 (m, 4H), 3.05 (t, 2H), 4.58 (s, 2H), 6.63 (brs, 2H), 7.59 (brs, 2H), 8.73 (s, 1H). Anal. Calcd. for $\text{C}_{11}\text{H}_{16}\text{N}_6\text{O}$: C, 53.21; H, 6.50; N, 33.85. Found C, 53.02; H, 6.51; N, 33.75.

2-Amino-6-n-butoxymethyl-4(3H)-pteridinone. mp > 300°C, UV (0.1 N NaOH) λ_{max} 255.5 (26,900), 2755.5 sh (10,600), 364 (7,400)nm; NMR ($\text{Me}_2\text{SO}-d_6$) δ 0.87 (t, 3H), 1.0-1.8 (m, 4H), 3.50 (t, 2H), 4.57 (s, 2H), 6.97 (brs, 2H), 8.67 (s, 1H) (ring NH exchanged out due to large amount of H_2O present); Anal. Calcd. for $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_2$ 0.4 H_2O : C, 51.51; H, 6.21; N, 27.31. Found: C, 51.45; H, 6.13; N, 27.35.

(+)-2-Amino-5,6,7,8-tetrahydro-6-n-butoxymethyl-4(3H)-pteridinone. mp 211-212°C dec; UV (0.1 N HCl) λ_{max} 265 (15,800)nm; NMR ($\text{Me}_2\text{SO}-d_6$) δ 0.88 (t, 3H), 1.0-1.7 (m, 4H), 3.0-3.8 (m, 7H), 4.95, 6.85, 7.45, 10.5 (br bumps). Anal. Calcd. for $\text{C}_{11}\text{H}_{19}\text{N}_5\text{O}_2$ 1.75 HCl. H_2O : C, 39.42; H, 6.84; N, 20.90; Cl, 18.52. Found: C, 39.40; H, 6.74; N, 21.06; Cl, 18.61.

Example 3: A mixture of 2-amino-7,8-dihydro-6-methoxymethyl-7,7-dimethyl-4(3H)pteridinone dihydrochloride

A mixture of 2-amino-7,8-dihydro-6-methoxymethyl-7,7 dimethyl 4(3H) pteridinone, 0.10g and platinum oxide (prereduced) 0.015 g, in 6 mL of 2N HCl was hydrogenated at 1 atm H_2 at room temperature for 2.5 hr; the mixture was filtered, and the filtrate was evaporated. This crude product was recrystallized from 6N HCl/MeCN to yield 2-amino-5,6,7,8-tetrahydro-6-methoxymethyl-7,7-dimethyl-4(3H)-pteridinone hydrochloride 0.089 g as a white solid, m.p. 214-300°C (dec), 76% of theoretical yield.

Elemental analysis: Calcd. for $\text{C}_{10}\text{H}_{17}\text{N}_5\text{O}_2$ 2HCl: C, 38.47, H, 6.13, N, 22.43; Cl, 22.71. Found C, 38.33; H, 6.16; N, 22.34; Cl, 22.71.

Examples 4 and 5

The following compounds were prepared from their corresponding precursors by the method described in example 3.

4. 2-Amino-5,6,7,8-tetrahydro-7-hydroxymethyl-6,7-dimethyl-4(3H)-pteridinone.
m.p. > 300°C.

Elemental analysis: Calcd. for $C_9H_{15}N_5O \cdot 1/20 C_9H_{13}N_5O \cdot 23/20 HCl \cdot 11/25 C_3H_8O \cdot 3/20 H_2O$: C, 42.37; H, 6.79, N, 23.85; Cl, 13.10. Found C, 42.41; H, 6.75; N, 23.89; Cl, 13.10.

5. 2-Amino-5,6,7,8-tetrahydro-6-hydroxymethyl-7,7-dimethyl-4(3H)-pteridinone dihydrochloride. m.p. 200°C (dec).

Elemental analysis: Calcd. for $C_9H_{15}N_5O_2 \cdot 2HCl$: C, 36.25; H, 5.75; N, 23.49; Cl, 23.78. Found: C, 36.13; H, 5.77; N, 23.42; Cl, 23.65.

Example 6: (+)-2-Amino-6-butoxymethyl-5,6,7,8-tetrahydro-7,7-dimethyl-4(3H)-pteridinone dihydrochloride hemihydrate

A solution of 2-amino-6-butoxymethyl-7,8-dihydro-7,7-dimethyl-4(3H)-pteridinone (153 mg, 0.548 mmol) in trifluoroacetic acid (3 ml) was hydrogenated over platinum oxide (11.3 mg) under 1 atm of H_2 at room temperature for 15 min. The product was worked up as before. Yield: 0.15 g (75%) of white powder: m.p. dec. above 200°C; IR (KBr) 2960, 2870, 1650, 1570, 1450, 1400, 1180, 1120, 735 cm^{-1} ; UV (0.1N HCl) λ_{max} : 217 nm (e) (19900), 263.5 (14300), λ_{min} : 239 (4000); ^1H-NMR (DMSO- d_6) δ from TMS: 0.88 (m, 3H, Me), 1.14 (s, 3H, Me), 1.28 (s, 3H, Me), 1.28 (s, 3H, Me), 1.44 (m, 4H, CH_2CH_2), 3.44 (m, 5H, C-6H, CH_2CH_2), rest exchanged.

Anal. Calc'd for $C_{13}H_{23}N_5O_2 \cdot 2HCl \cdot H_2O$:

C, 42.98; H, 7.21; N, 19.28; Cl, 19.52.

Observed: C, 42.95; H, 7.22; N, 19.48; Cl, 19.51.

Using a method analogous to that described in Example 6 the following compounds were prepared.

Example 7: (+)-2-Amino-5,6,7,8-tetrahydro-6-isobutoxymethyl-7,7-dimethyl-4(3H)-pteridinone

RSB/KMS/3375/19.08.83

dihydrochloride

M.p. dec. above 190°C

Anal. calc'd. for $C_{14}H_{25}N_5O_2 \cdot 2 HCl$:

C, 45.65; H, 7.39; N, 19.02; Cl, 19.25. Observed: C, 45.53; H, 7.40; N, 19.00; Cl, 19.11.

Example 8: (+,-)-2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-6-octyloxymethyl-4(3H)-pteridinone dihydrochloride

M.p. dec. above 200°C

Anal. calc'd. for $C_{17}H_{31}N_5O_2 \cdot 2HCl$:

C, 49.75; H, 8.10; N, 17.07; Cl, 17.28.

Observed: C, 49.68; H, 8.13; N, 17.07; Cl, 17.26.

Example 9: (+,-)-2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-6-pentoxymethyl-4(3H)-pteridinone dihydrochloride

M.p. dec. above 190°C

Anal. calc'd for $C_{14}H_{25}N_5O_2 \cdot 2 HCl$:

C, 45.65; H, 7.39; N, 19.02; Cl, 19.25.

Observed: C, 45.63; H, 7.43; N, 19.02; Cl 19.19.

Example 10: (+,-)-2-Amino-6-(3-cyclohexylpropoxymethyl)-5,6,7,8-tetrahydro-7,7-dimethyl-4(3H)-pteridinone dihydrochloride

M.p. = 216-221°C (dec)

Anal. calc'd for $C_{19}H_{31}N_5O_2 \cdot 2 HCl$:

C, 51.18; H, 7.87; N, 16.58; Cl 16.79.

Observed: C, 50.97; H, 7.91; N, 16.52; Cl, 16.33.

Example 11: (+,-)-2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-6-(3-phenylpropoxymethyl)-4(3H)-pteridinone dihydrochloride

M.p. = 194-200°C (dec).

Anal. calc'd for $C_{18}H_{25}N_5O_2 \cdot 2 HCl$

C, 51.92; H, 6.54; N, 16.82; Cl 17.03

Observed: C, 51.73; H, 6.57; N, 16.72; Cl, 17.03.

Example 12: (+,-)-2-Amino-5,6,7,8-tetrahydro-6-isopropoxymethyl-7,7-dimethyl-4(3H)-pteridinone dihydrochloride one-quarter hydrate

M.p. = 190-192°C (dec)

Anal. calc'd. for $C_{12}H_{21}N_5O_2 \cdot 2 HCl \cdot \frac{1}{4} H_2O$:

C, 41.81; H, 6.87; N, 20.31; Cl, 20.57.

Observed: C, 41.79; H, 6.89; N, 20.27; Cl, 20.59.

Example 13: (+,-)-2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-6-propoxymethyl-4(3H)-pteridinone dihydrochloride

Anal. calc'd for $C_{12}H_{21}N_5O_2 \cdot 2 HCl$:

C, 42.36; H, 6.31; N, 20.58.

Observed: C, 42.31; H, 6.81; N, 20.53.

Example 14: (+,-)-2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-6-propyl-4(3H)-pteridinone dihydrochloride

UV (0.1N HCl) λ_{\max} : 263.5, 214 nm; λ_{\min} : 238.5 nm.

Example 15: (+,-)-2-Amino-5,6,7,8-tetrahydro-6-isopropyl-7,7-dimethyl-4(3H)-pteridinone dihydrochloride

UV (0.1N HCl) λ_{\max} : 264, 217 nm; λ_{\min} : 239 nm.

Example 16: (+,-)-2-Amino-6-ethyl-5,6,7,8-tetrahydro-7,7-dimethyl-4(3H)-pteridinone dihydrochloride hemihydrate

Anal. calc'd for $C_{10}H_{17}N_5O \cdot 2 HCl \cdot \frac{1}{2} H_2O$:

C, 39.35; H, 6.60; N, 22.95; Cl, 23.23.

Observed: C, 39.11; H, 6.51; N, 22.95; Cl 23.26.

Example 17: (+,-)-2-Amino-6-ethoxymethyl-5,6,7,8-tetrahydro-7,7-dimethyl-4(3H)-pteridinone dihydrochloride 3/4 hydrate

M.p. = 203-207°C (dec)

Anal. calc'd for $C_{11}H_{19}N_5O_2 \cdot 2 HCl \cdot \frac{3}{4} H_2O$:

C, 38.89; H, 6.68; N, 20.61; 20.87.

Observed: C, 38.92; H, 6.64; N, 20.72; Cl, 20.77.

Example 18 Biological Data

Tyrosine hydroxylase was partially purified through a 25-45% ammonium sulfate fraction. Specific activity of this preparation with tetrahydrobiopterin was 4.0 nmole/mg protein (min). the enzyme was assayed by a modification of the method of Nagatsu, T., Levitt, M., and Udenfriend, S. (Anal. Biochem. 9, 122, (1964)) where the Dowex chromatography step was replaced by a charcoal extraction.

Tryptophan hydroxylase was assayed according to a modification of the method of Renson J., et al (Biochem. Biophys. Acta 25: 504 (1966)). The preparation of tryptophan hydroxylase was a crude 30,000xg supernatant which had been desalted on a Sephadex G-25 (Trade Name) column (Boadle-Biker, M.C., Biochem Pharmacol. 28: 2129 (1979)). The specific activity of this tryptophan hydroxylase, using BH_4 as cofactor, was approximately 100 pmol product per milligram protein per minute at 37° .

Phenylalanine hydroxylase was measured as described by Shiman, R. et al., (J. Biol. Chem. 254: 11300 (1979)) except that the product of the reaction (tyrosine) was measured fluorometrically using the method of Waalkes, T.P. and Udenfriend, S. (J. Lab. Clin. Med. 50: 733 (1957)). Phenylalanine hydroxylase was prepared from rat liver using hydrophobic chromatography (Shiman, R. et al. (J. Biol. Chem. 254: 11,300 (1979)). The specific activity of the enzyme, using BH_4 as the cofactor, was $1.0 \mu\text{mol/mg protein per minute}$ at 37° . Dihydropteridine reduction was assayed by the method of Crane, et al (J. Biol. Chem., 247, 6082 (1972)).

Table I
Biological Activity of Various Pterins

| Compound of Formula (I) | R ² | R ³ | R ⁴ | V _{max} (%) Tyrosine Hydroxylase | Tryptophan Hydroxylase | Phenylalanine Hydroxylase | Dihydro peridine reductase |
|---|----------------|-----------------|--------------------|---|---------------------------|------------------------------|-------------------------------|
| Tetrahydrobiopterin (BH ₄) | | | | 100 | 100 | 100 | 100 |
| CH ₂ -O-CH ₃ , | H, | CH ₃ | CH ₃ | 138 | 0 | 5 | 316 |
| CH ₂ OH, | H, | CH ₃ | CH ₃ | 102 | 0 | 5 | 161 |
| CH ₃ , | H, | CH ₃ | CH ₃ | 54 | 0 | 43 | 316 |
| CH ₃ , | H, | CH ₃ | CH ₂ OH | 25 | 0 | 3 | 81 |
| H, | H, | CH ₃ | CH ₃ | 83 | 0 | 5 | 277 |
| CH ₂ OCH ₃ | H, | H | H | 124 | 54 | 79 | 295 |

Numbers are percent of maximal velocities, obtained with BH₄. Enzyme assays were performed as described in the text.

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20.

Example 19: Pharmaceutical FormulationsA. Capsule

| <u>Ingredient</u> | <u>Amount per capsule (mg)</u> |
|-------------------|--------------------------------|
| Compound I | 325.0 |
| Ascorbic Acid | 174.0 |
| Corn Starch | 100 mg |
| Stearic Acid | 27 mg |

The finely ground active compound was mixed with the powdered excipients lactose, corn starch and stearic acid and packed into a gelatin capsule.

B. Tablet

| <u>Ingredient</u> | <u>Amount per tablet (mg)</u> |
|----------------------|-------------------------------|
| Compound I | 325.0 |
| Ascorbic Acid | 125.0 |
| Corn Starch | 50.0 |
| Polyvinylpyrrolidone | 3.0 |
| Stearic Acid | 1.0 |
| Magnesium stearate | 1.0 |

The active compound was finely ground and intimately mixed with the powdered excipients lactose, corn starch, polyvinylpyrrolidone, magnesium stearate and stearic acid. The formulation was then compressed to afford one tablet weighing 505 mg.

A. Suppository

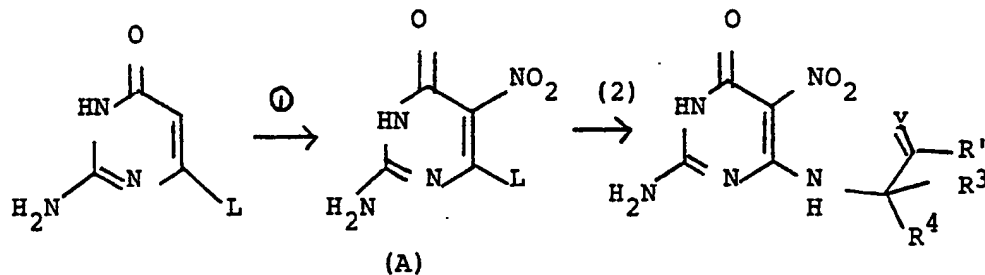
| <u>Ingredient</u> | <u>Amount per suppository</u> |
|-----------------------------------|-------------------------------|
| Compound I | 325.0 mg |
| Butylated hydroxy toluene (BHT) | 25 mg |
| Cocoa Butter or Wecobee* Base q.s | 2.0 g |

*Wecobee is the trade name of a hydrogenated carboxylic acid.

Scheme 1

Representative synthetic Routes to Compounds of the Formula IV.

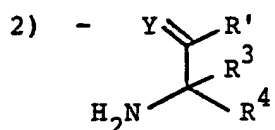
1) .



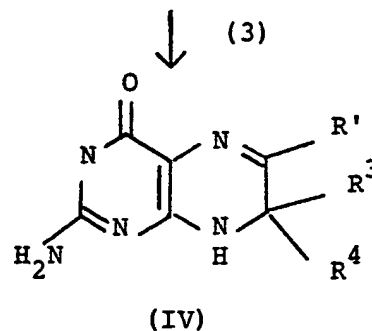
L = Leaving Group

Y = O, NOR, S

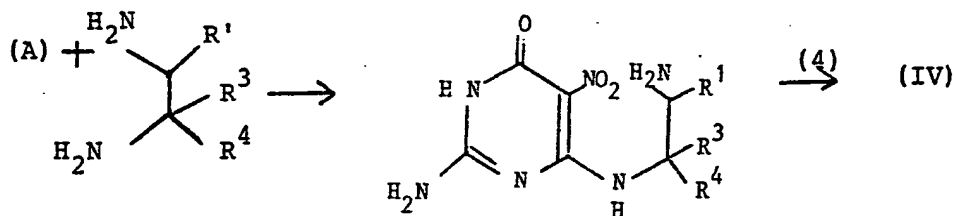
1) $-\text{HNO}_3, \text{H}_2\text{SO}_4$



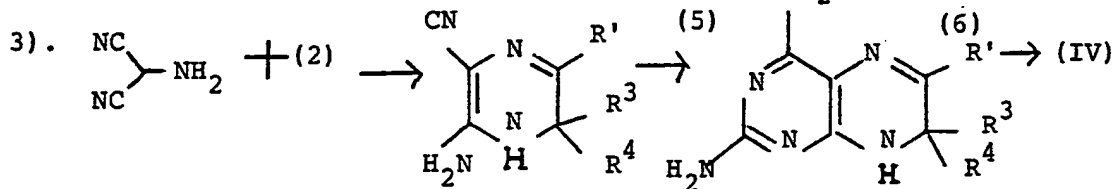
3) [H]



2) .



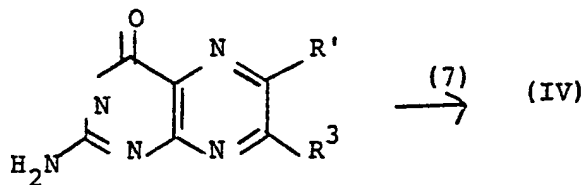
(4) heat or [H] followed by O_2



(5) - guanidine

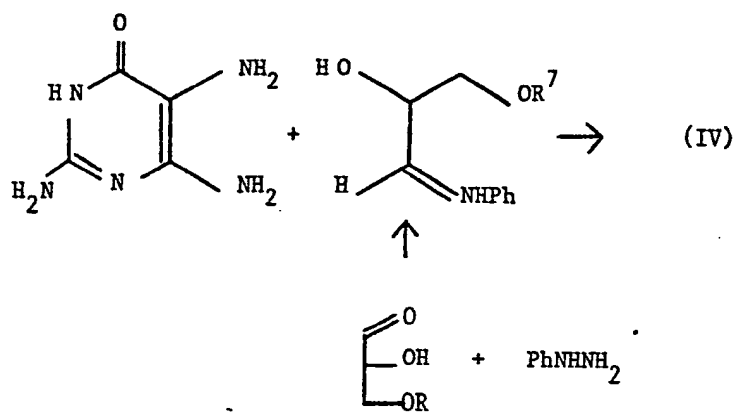
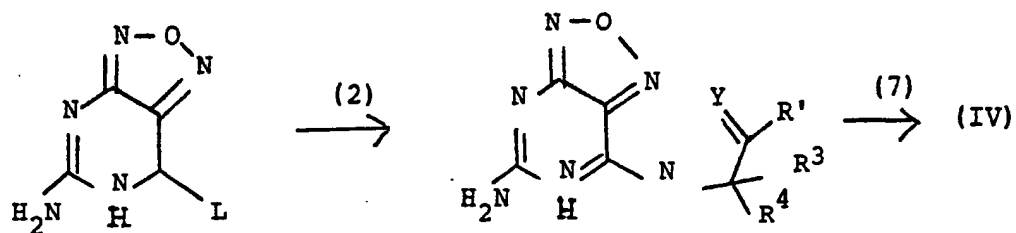
(6) - NaOH

4) .



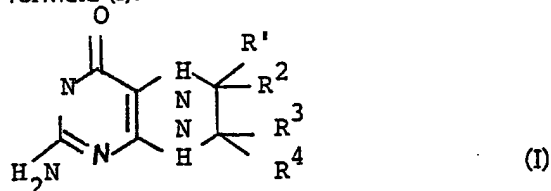
(7) - [H]

).



Claims

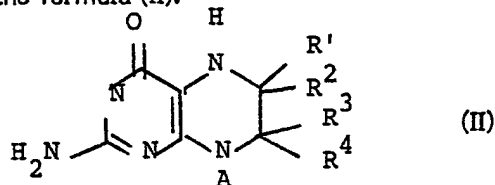
- 1.) A compound of the formula (I):



or a pharmaceutically acceptable salt or a bio-precursor thereof wherein R^1 is hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, a group $-BX(R^5)_n$, or $-BZBX(R^5)_n$ wherein Z and X (when $n=1$) are the same or different and each is $-O$, $-S(O)_q$ or $-NR^6$ wherein q is 0, 1 or 2 and R^6 is hydrogen or C_{1-4} alkyl or, when $n=0$, X is halogen; R^5 is hydrogen, C_{1-12} aralkyl or C_{1-10} alkyl; B is C_{1-5} alkanyl; R^2 is hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl or a group BOR^7 wherein B is as hereinbefore defined and R^7 is hydrogen or C_{1-6} alkyl; or R^1 and R^2 together with the carbon atom in the pteridine ring structure to which they are attached, form a C_{3-7} spirocycloalkyl ring; R^3 and R^4 are hydrogen or methyl; or R^2 and R^3 together with the carbon atoms in the pteridine ring structure to which they are attached form a C_{5-7} cycloalkyl ring; provided that at least one of R^1 , R^2 , R^3 and R^4 is hydrogen and that one of R^1 , R^2 and R^3 , R^4 represents gem di-substitution unless R^1 represents $-CH_2OR^7$ wherein R^7 is as hereinbefore defined in which case R^2 , R^3 and R^4 can all be hydrogen;

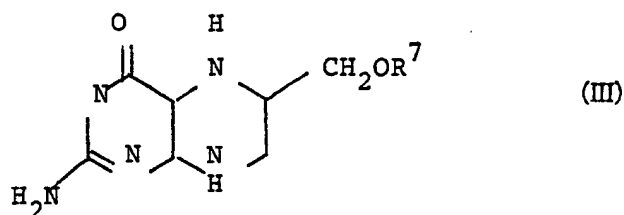
for use in medicine

- 2.) A compound of the formula (II):



or a pharmaceutically acceptable salt or bioprecursor thereof; wherein R^1 to R^4 are as hereinbefore defined and at least one of R^1 , R^2 and R^3 , R^4 represents gem di-substitution; for use in medicine.

3). A compound of the formula (III):



or a pharmaceutically acceptable salt or bioprecursor thereof;
wherein R^7 is as hereinbefore defined;
for use in medicine.

- 4). A compound of the formula (I) or a pharmaceutically acceptable salt thereof, provided that when R^3 and R^4 are methyl neither R^1 nor R^2 are methyl.
- 5). A compound of the formula (II), or a pharmaceutically acceptable salt thereof, as hereinbefore defined, provided that when R^3 and R^4 are methyl neither R^1 nor R^2 are methyl.
- 6). A compound of the formula (III), or a pharmaceutically acceptable salt or bio-precursor thereof, as hereinbefore defined.
- 7). A compound according to claim 4 or 5 wherein B is CH_2 , X is oxygen and $(R^5)_n$ represents hydrogen or a C_{1-10} alkyl group.
- 8). A pharmaceutical composition which comprises a compound of the formula (I) or a pharmaceutically acceptable salt thereof, as hereinbefore defined, together with one or more pharmaceutically acceptable carriers.
- 9). A pharmaceutical composition according to claim 8 which contains other active therapeutic ingredients.
- 10). An analogy method for the preparation of a compound of the formula (I) as defined according to claim 4.